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ELECTROPHORETIC SEPARATOR FOR PURIFYING BIOLOGICALS

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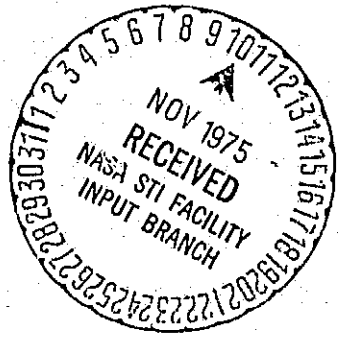

SUMMARY

During the first year of effort on this contract the mathematical expressions were developed to describe the interrelationships between operating requirements (capabilities), cell parameters, and system constraints in terms of design criteria definition. The mathematical model was programmed for computer solution. The model was exercised to identify performance-limiting characteristics, and analyses were conducted to predict operation in space of an experiment involving separation of four components.

An engineering model of a flowing electrophoretic separator has been constructed. The design is directed toward verifying improvements in resolution and throughput of a thicker cell than can be used on earth.

As further designs evolve, the capability of processing selected significant biologicals may be tested and proven. These may include means to recover sample fractions and a UV scanner for detecting them.

Of the three major tasks included in this contractual effort, Task I, Design Criteria Optimization-Math Modeling, is complete except for a recently-discovered minor problem which will be corrected soon. Task II, Design, Build, and Test a Flowing Electrophoretic Separator System, is

nearly complete. The system has been designed and built. Testing of the system is just beginning. Task III, Experimental Operations Task, has been started. By use of prototype equipment similar to that designed in Task II, assessments are being made of the effects of cell thickness, voltage gradients, buffer conductivity, residence differences, and concentrations.

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I. INTRODUCTION

One of the most promising ideas identified for space processing of materials is the separation and/or purification of biological materials by electrophoresis. Although electrophoresis was first observed in the early nineteenth century, its utilization as a laboratory technique did not take place until the 1930's when precise measurements were made on purified proteins and complex biological materials. Most of the development in electrophoresis since 1950 has been in an area described as zone electrophoresis in stabilized media. This technique separates a single narrow zone of sample mixture in an electrolyte medium into many zones containing a single component of the mixture and electrolyte between them. Since the densities of the separated zones generally differ from that of the intervening medium, such systems are gravitationally unstable and stabilization is required. The various techniques for stabilization include using the capillary spaces provided by thin films, the interstices of solid material such as filter paper and a variety of gel-forming substances.

Although zone electrophoresis has been adapted for both continuous and batch fractionation, much care and ingenuity have been involved in the choice and preparation of the stabilizing medium. This method is usually satisfactory for separating low molecular weight materials but it is impractical for high molecular weight materials because of immobility in the stabilizing media, and interactions between the macroion and the stationary phase.

Attempts have been made to use fluid electrophoresis for the separation of high molecular weight materials which have little or no mobility in a porous solid. In the presence of gravity, however, the method is characterized by incomplete separations, due to electrophoretic and hydrodynamic effects near the cell wall, and to density differences between the solvent and the solute. In addition, large-scale separations are limited by thermal convection arising from Joule heating of the solution.

Electrophoresis done in space will alleviate at least two major problems that occur on earth. (1) The electric field produces an electric current in the liquid medium which results in Joule heating. This heating generates convection currents in the solution which mix the components already separated. (2) Large biological particles of high density, such as living cells, settle to the bottom of liquid electrophoresis beds and cannot be effectively separated. Under weightless conditions, electrophoresis can be applied to molecules or particles of any size or density suspended in fluid media. The advantages are expected to make electrophoretic separation in space practical for preparing medical and biological products of high social and economic value.

The objectives of this project are:

1) to develop the analytical and experimental basis for a thick cell, free-flow electrophoretic separator for application in the low gravity of space;

2) to design, build, and test an engineering model of the electrophoretic separator;

3) to demonstrate the experiment operation on the ground with simulated weightless conditions;

4) to operate the model and conduct experimental simulation studies to provide data and experience that will predict the performance of the equipment in the space environment;

5) to predict the operation in space based upon analysis and experimentation and identify problem areas that remain unresolved by ground evaluation that must be tested or measured in space; and

6) to define flight experiments of varying duration that will demonstrate the performance of the design and apparatus concepts.

II. MATHEMATICAL MODEL OF AN ELECTROPHORETIC SEPARATOR

A. Background

Under an earlier contract (NAS 8-28365) an equation was derived which relates to various parameters the minimum difference in mobility ($\Delta\mu$) necessary to separate two particles in an electrophoresis device.

The equation previously derived for $\Delta\mu$ is:

$$\Delta\mu\ \% = \frac{\Delta S_1 + N + bs}{S_1} \times 100$$

S_1 is the horizontal displacement, ΔS_1 is distortion of the sample, N is the spacing between cell outlets, and b_s is half the sample stream diameter.

The solution of such an equation normally involves a number of simplifying assumptions which are made necessary by the interdependence of several of the parameters implicit to the solution. Therefore, in order to obtain more realistic estimates of electrophoretic resolving power, a computer program was developed which did not make use of simplifying assumptions but rather took into account the various thermal, electrical, and hydrodynamic effects and their interrelationships.

B. Description of the Computer Model

The computer model provides a total view of the interacting variables in the electrophoretic system. By altering these variables, it is possible to determine the effect on a given separation.

The total model is composed of several individual programs, each of which solves a particular part of the problem. In this way, flexibility is provided and alterations to individual parts can be made without interfering with the operation of any of the other parts.

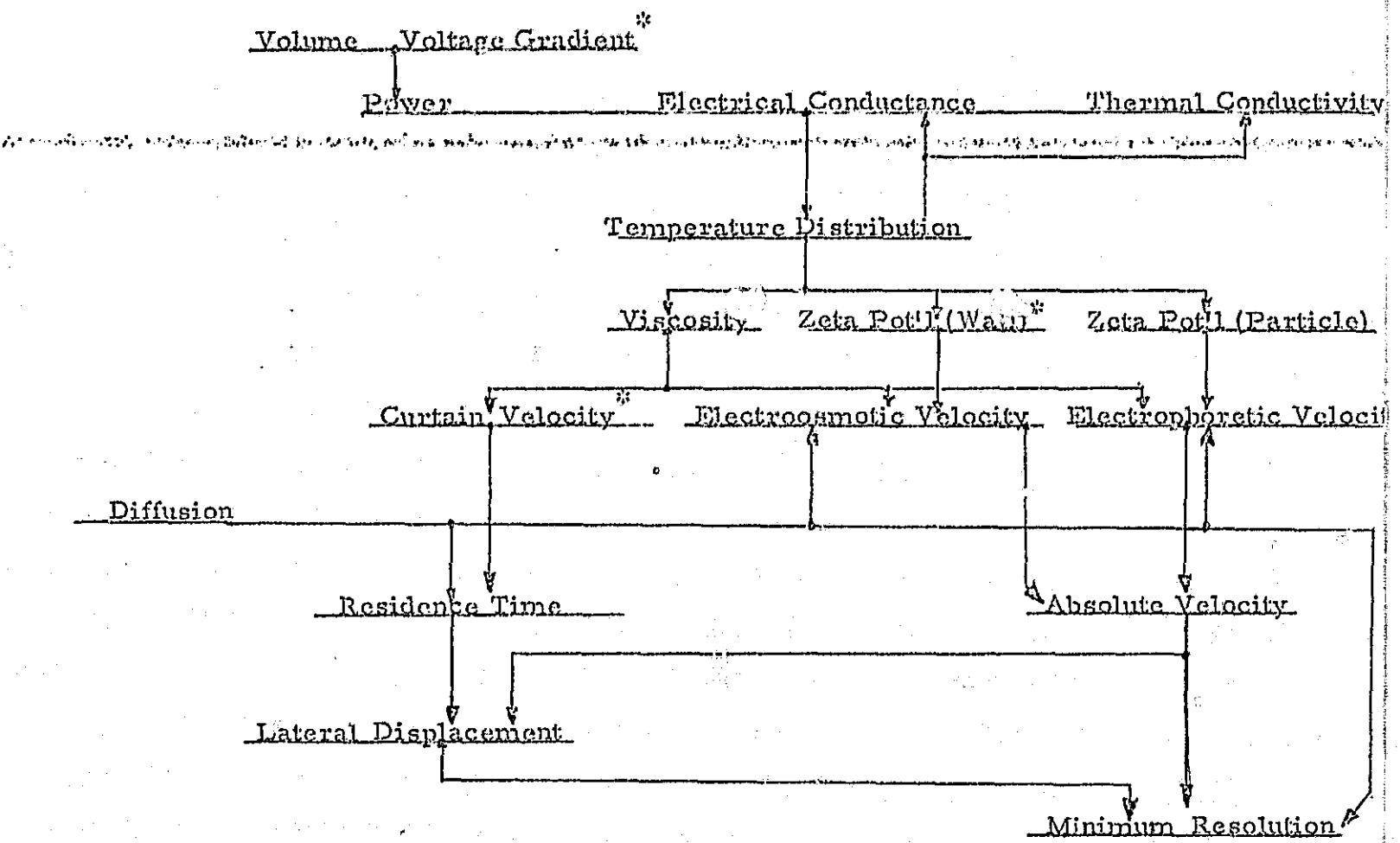
Although each part of the model is independent from all others, the results of each part are used in subsequent programs. Figure 1 illustrates the interdependence of the cell's variables.

Step 1: Temperature Distribution

An equation was set up to describe the temperature gradient though the thickness of the electrophoresis cell. The equation was set up for the condition that both faces of the cell are at the same temperature and a steady-state condition has been reached. (This, of course, would be normal operating procedure.) The calculations yield a temperature difference, θ, from the temperature of the outside face. These distributions are therefore symmetrical. Some standard, textbook equations (Ref. 1) were solved first to acquire a "feel" for the solutions. The final equation

(1). Jakob, M., "Heat Transfer, Vol. 1", J. Wiley & Sons, New York, N.Y., 1949.

FIGURE 1. INTERDEPENDENCE OF CELL VARIABLES



* Quantities Easily Controlled

is a second order differential equation which takes into account variations in thermal conductivity, k, and electrical conductivity, ke, with temperature. Both of these variables increase with temperature over the range of interest (4°C to 37°C). Both vary according to an equation of the form: $\chi = \chi_s (1 + a\theta)$ where χ_s is the quantity at a standard temperature, a is the temperature coefficient and θ is the temperature difference. The final equation has the form:

$$(a + \delta\theta)\frac{d^2\theta}{dx^2} + \delta\left(\frac{d\theta}{dx}\right)^2 = -\beta_s'''(1+\beta\theta) \qquad (1)$$

where a is the thermal conductivity at 4°C, δ is the temperature coefficient of the thermal conductivity, q'''_s is the heat generated per unit volume and contains both the voltage gradient and the electrical conductivity, β is the temperature coefficient of q'''_s, θ is the temperature difference and x is the distance from the centerline of the cell.

This equation is solved by the computer in Step 1. The data are obtained as a profile of temperature difference versus distance from cell centerline. In addition, this printout indicates when temperature will exceed physiological temperature of 37°C.

Inputs to Step 1 are: buffer conductance and its temperature coefficient, thermal conductivity and its temperature coefficient, voltage gradient and one-half the cell thickness.

Step II: Curtain Velocity Profile

The linear velocity of the buffer curtain determines the residence time of a particle in the field and therefore determines, in part, the lateral displacement of the particle. In a constant temperature system, the velocity profile would be parabolic, due only to viscous friction. However, viscosity decreases with increasing temperature (for a liquid) and since there is a distribution of temperature in the cell, a distortion of the parabolic flow profile results.

The final equation for Step II is also a second order differential equation of the form:

$$\frac{\partial}{\partial x}\left\{\left(\eta \frac{\partial V}{\partial x}\right)\right\} = \frac{dP}{dy} \qquad (2)$$

where η is the viscosity of the buffer, dP/dy is the pressure gradient causing flow, V is the velocity of the buffer at x, the distance from the cell centerline.

It is important to know the velocity at all points through the cell thickness, since a sample stream has a finite thickness and therefore particles at the outer edge of the stream will move with a lower velocity than particles at the center. The slower parts of the stream have longer residence times and therefore experience different lateral displacement. This ultimately affects resolution.

Inputs to Step II are: average flow rate, cell width, cell thickness, temperature data from Step I and the variation of viscosity with temperature.

Step III: Profile of Electroosmotic Velocity

Electroosmosis occurs normal to the direction of hydrodynamic flow. Since the cell is a closed system in the electroosmotic direction, this flow must be recirculating. Depending upon the sign and magnitude of the applied field and the zeta potential at the wall, this electroosmotic flow will affect the lateral displacement of a particle undergoing electrophoresis. It is necessary to know the profile of this flow, so that an absolute velocity can be calculated for particles at various positions in the cell. The final equation is almost identical to that of Step II except for substitution of -F for dP/dy.

$$\frac{d}{dx}\left\{\eta \frac{dVe}{dx}\right\} = -F \qquad (3)$$

where η is again viscosity, Ve is the electroosmotic velocity, -F is the driving force for flow related to the zeta potential of the wall and x is a distance from the cell centerline.

Inputs to Step III are: Zeta potential of the wall surface, dielectric constant of the buffer, field gradient, cell thickness, temperature data from Step I and variation of viscosity with temperature.

Step IV - Final Residence Time and Diffusion Effects

Depending on the kinds of particles in the sample stream and on the residence time, diffusion effects may play an important role in the separation and resolution achieved. Diffusion will cause the sample stream to widen as it transverses the cell. If the diffusion time is short compared to the residence time, sample particles will be moving into slower curtains and the residence times will thus increase. This has to be taken into account when computing the final residence time. The effect of diffusing into a slower stream can be compared to a decelerating force and the increase in residence time can be calculated by using the following equation:

$$S = V_o tr - 1/2\ a\ tr^2 \qquad (4).$$

where S is the length of the field in the cell, V_o is the initial velocity of the particle, α is the change in velocity due to diffusion and tr is the residence time.

Inputs to Step IV are: active length of cell, centerline velocity of particle stream (from Step II) deceleration factor, α, from Step II and the diffusion constants of the particles.

Steps V-VII - Total Lateral Displacement

The total lateral displacement of a particle in the field is a result of electrophoresis, electroosmosis and residence time. Steps V through VII calculate the electrophoretic velocities and the electroosmotic velocities throughout the thickness of the sample stream and arrive at net velocities for the particles. Combining these with their respective residence times allows calculation of total lateral displacement, taking into account temperature variations.

Step VIII - Minimum Resolution

It is necessary to define a minimum resolution (distance between two sample streams) for complete separation. This minimum resolution takes into account lateral displacement for two particles, collection tube spacing, particle stream diameters and increases in these due to diffusion effects.

The result is a number which is the minimum difference in mobility between two particles which is necessary to achieve a complete separation.

It is possible to modify Step VIII so that if the mobility difference is already known, it is possible to calculate any of the other variables. Thus the operating parameters can be optimized for a sample of a known composition.

C. Use of the Computer Model

Some test cases were run on the computer program with input values derived from our own experience or reported by others. The input data set included:

Cell dimensions	active length	10.16 cm
	width	5.08 cm
	thickness	0.50 cm
Field	40 volts/cm	
Electrical Conductivity	(A-1 buffer)	8.75×10^{-4} ohm^{-1} cm^{-1}
	Temp. Coeff.	2.88% $^{o}C^{-1}$
Thermal Conductivity		1.36×10^{-3} cal/sec/cm/^{o}C
	Temp. Coeff.	4.05×10^{-6} $^{o}C^{-1}$
Buffer Flow Rate	1.05 cm^3/min	
Sample Diffusion Coeff.	$5. \times 10^{-8}$	Zeta Potential Sample (Particle.
Sample Stream Radius	0.03 cm	1) 0.025 2) 0.029 3) 0.031 4) 0.033 } Volts
Collector Spacing	0.1 cm	

Two cases were considered, one where the cell wall was 0.005 volts, and one where it was 0.050 volts. The first approximates an agarose-coated wall; the second a 1:1 agarose:agar-coated wall. Plots of some of the data are shown in Figures 2 through 5.

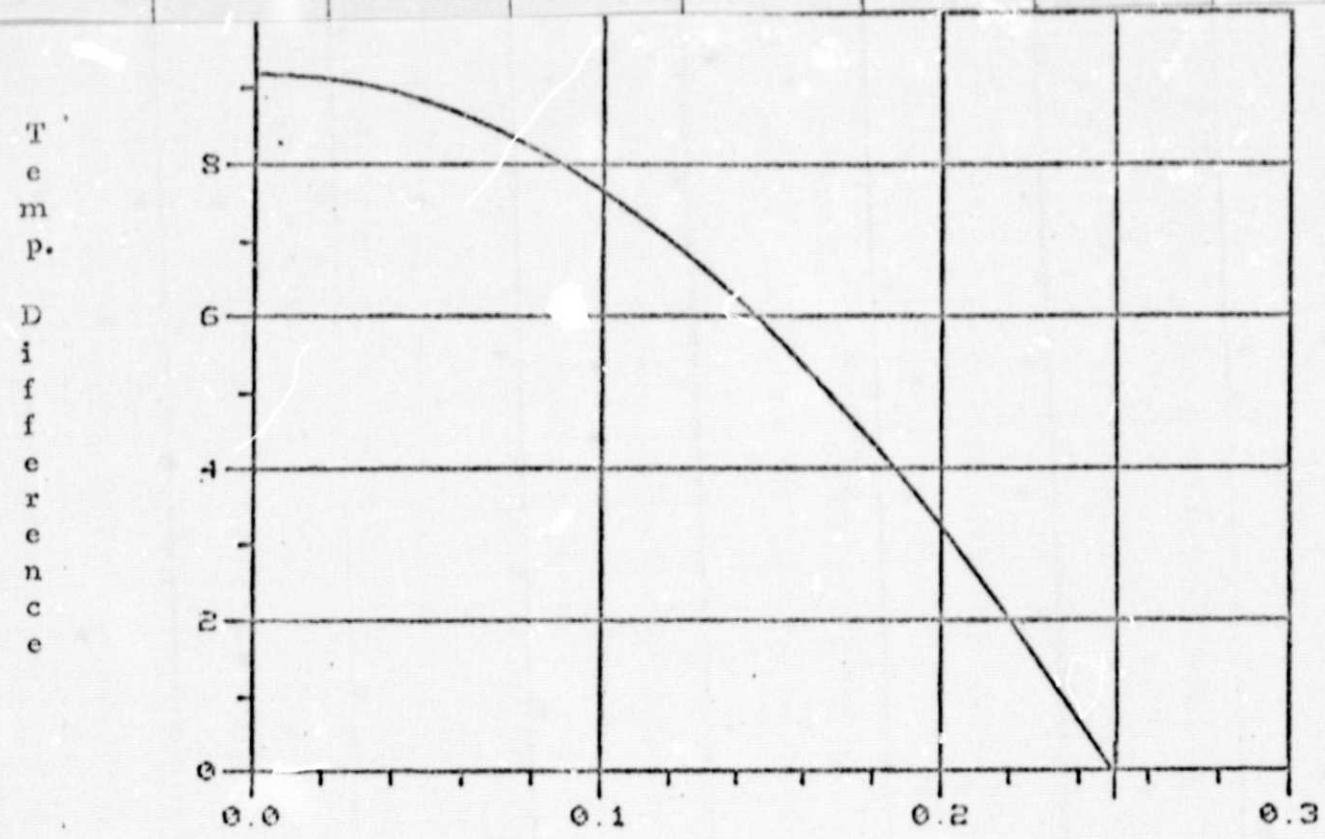


Figure 2. Temperature Gradient vs. Distance from Center-Line.

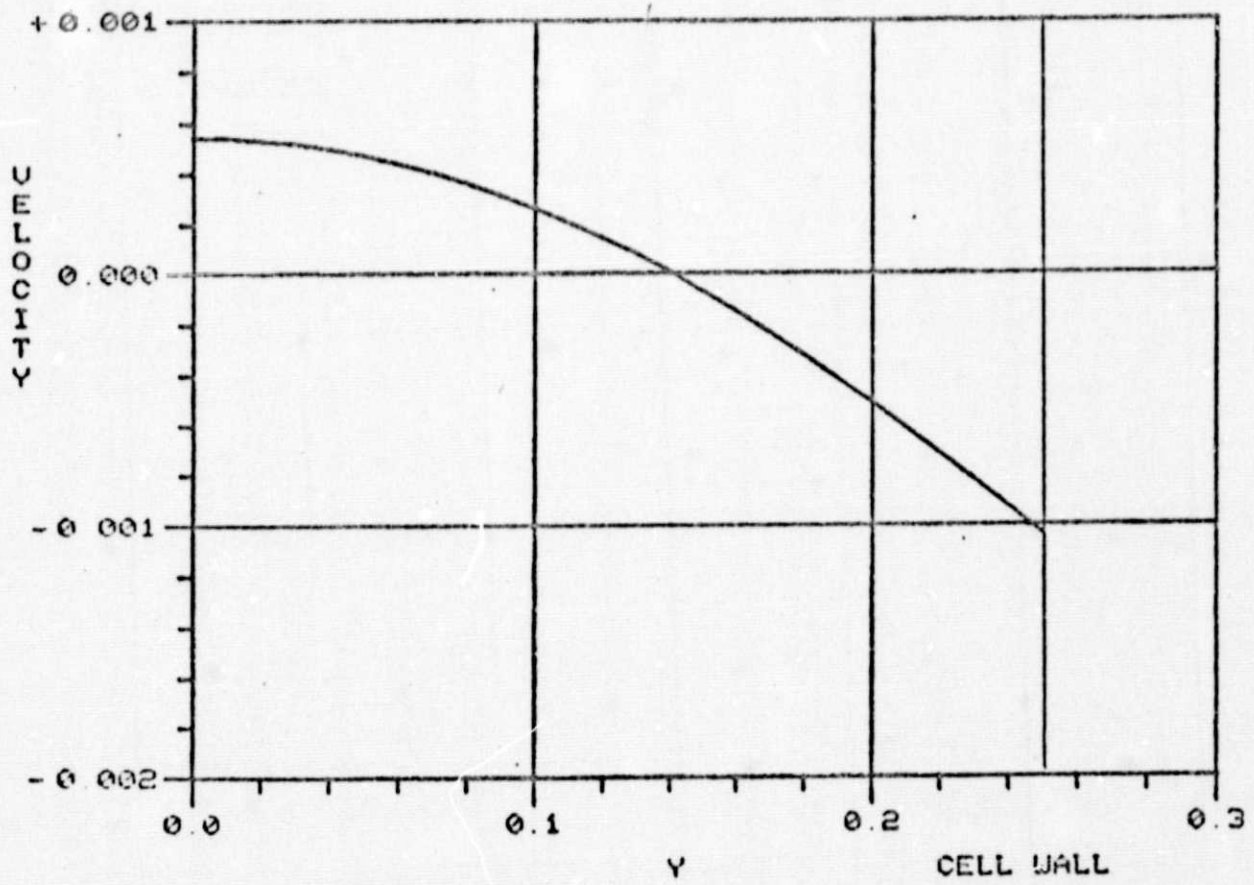


Figure 3. Electro-Osmosis Profile.

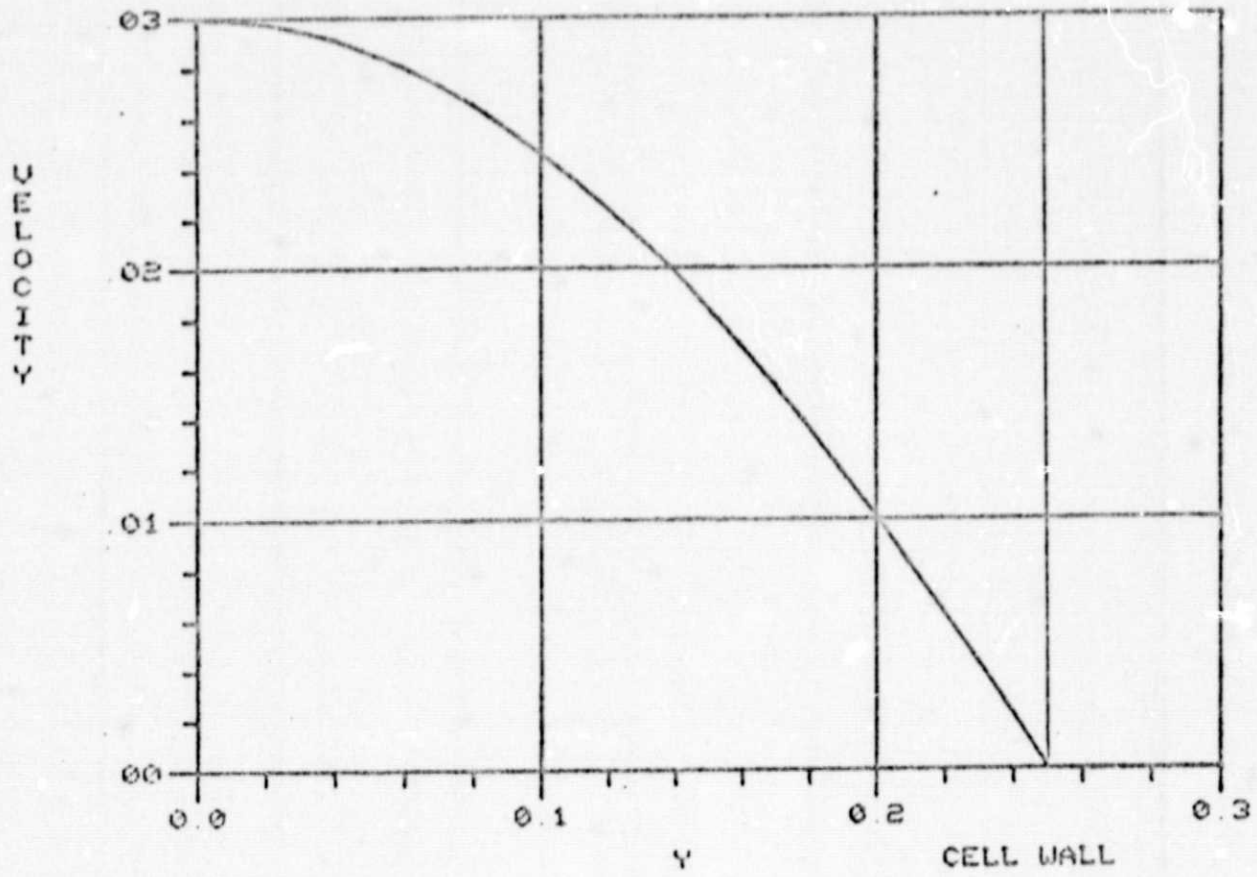


Figure 4. Buffer Velocity Profile.

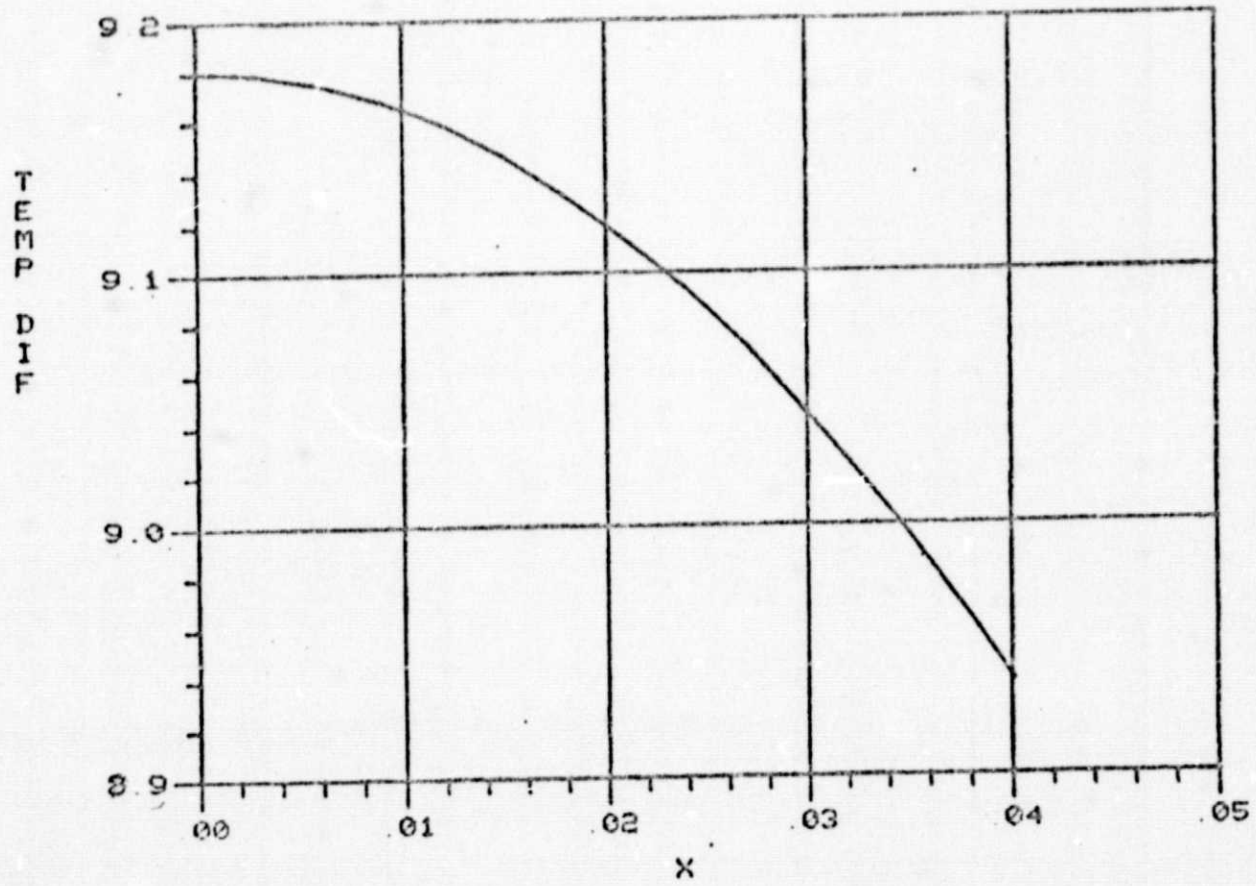


Figure 5. Temperature vs. Distance from Center of Sample Stream

Minimum values of $\Delta\mu$ % (see Section IIA, above) were calculated for the several cases and are summarized in Table 1. The results indicate quite clearly the advantage of using an agarose/agar coating on the cell walls when attempting to separate particles of the types being considered. We have recently learned that under some circumstances the program does not give exactly the correct answers. For example, the data shown in Table 1 for the case of the 0.05 volt wall zeta potential are close but not exactly correct. We are trying to determine the cause of this anomaly.

TABLE 1

Minimum Mobility Differences Required for Separation

Sample Particle Zeta Potential, volts	$\Delta\mu$ %	
	Wall ZP .005 volts	Wall ZP .050 volts
.025	7.54	1.99
.029	6.78	1.97
.031	6.56	1.96
.033	6.25	1.95

III. ENGINEERING MODEL OF THE ELECTROPHORETIC SEPARATOR

A. Summary

An engineering model of a free flow electrophoretic separator and the test and operating panel for running it were designed, assembled, and are now undergoing system tests. It is sized and designed to fit on the Black Brant sounding rocket for testing under zero g conditions. It is 14.44" dia. x 19" high and weighs about 76 lbs.

The engineering model is shown in Figure 6 with the air-tight cover removed. It is shown alongside the ground operation and test panel. Figure 7 is a view of the equipment with the cover on but with the access door open as it would be for last-minute adjustments, addition of sample, or removal of film or sample after flight. Also visible in these views are connectors which include the means for testing the entire unit as well as for power and telemetry operations during flight. In addition the fluid connectors permit the preflight filling and flushing of the chilling fluid to freeze the temperature controllant, the buffer system, and the general atmosphere of the unit which is to be dry nitrogen.

The unit is designed to provide both a wide range of current and future capabilities. Initial capabilities are to provide photographic data acquisition from a cross-section analyzer to indicate resolution in the 5 mm thick cell. The reusable unit is equipped to operate over a wide range of conditions including: temperatures down to 4°C for several hours to preserve delicate biologicals, 0 to 150 volts/cm and 0-200 ml/min. of buffer flow

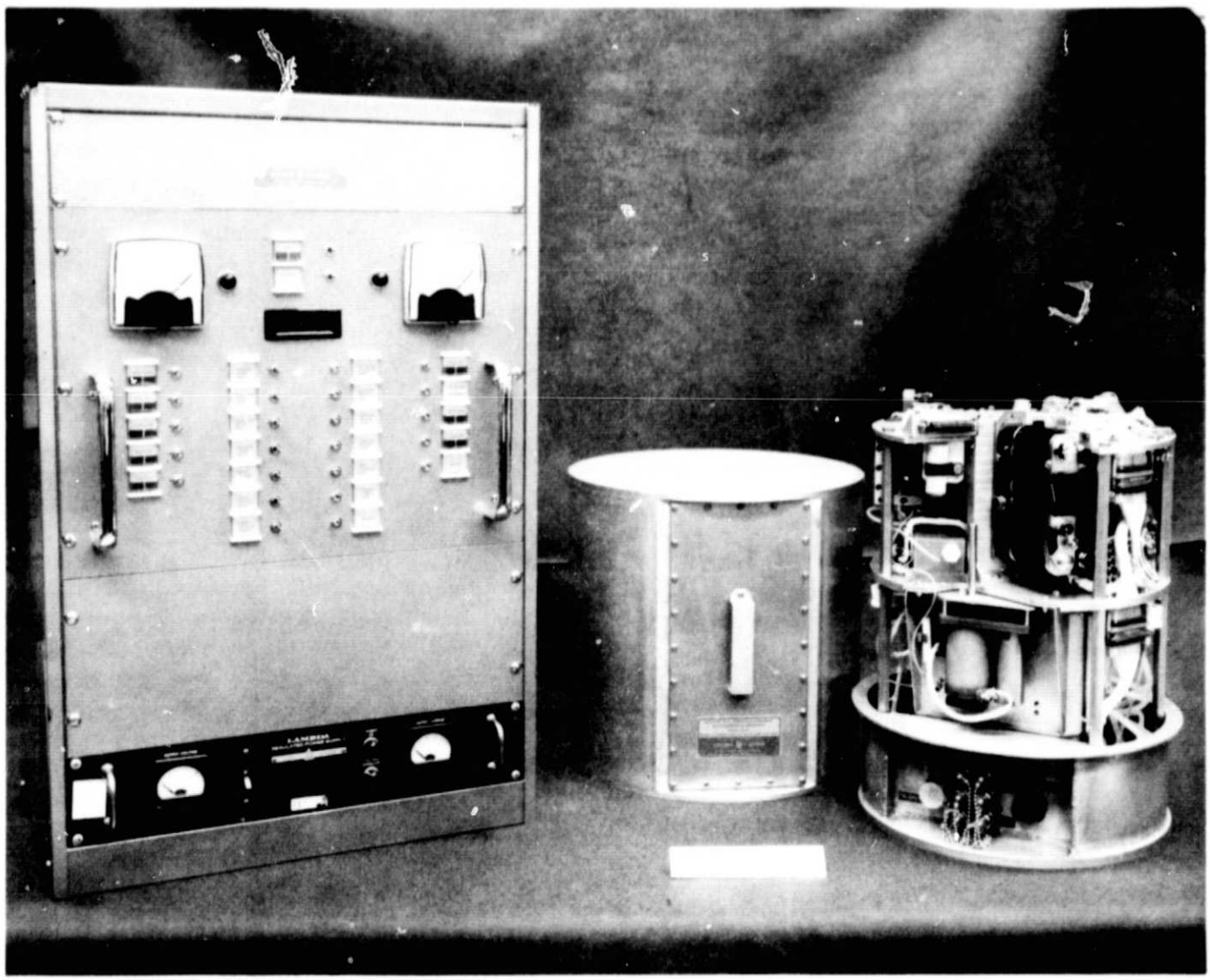


Figure 6. Engineering Model of Electrophoretic Separator with Control Panel.

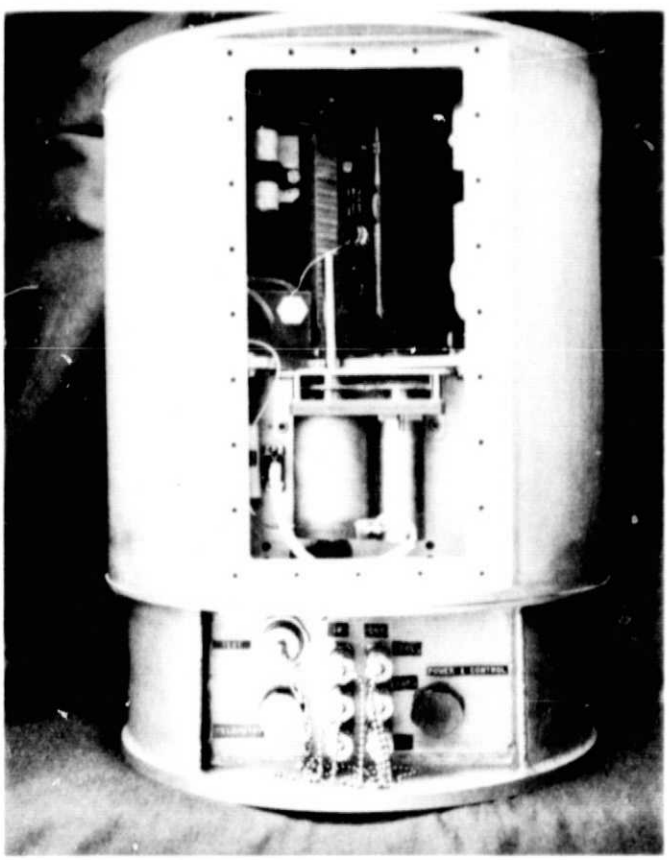


Figure 7. Engineering Model of Electrophoretic Separator with Access Door Open

for up to about 8-15 minutes depending upon the flow rate chosen. This is to permit operation on the Aries rocket if desired. In the latter case, this includes the ability to function as a static cell in which the sample can be inserted by flowing the buffer and sample into the field area, then stopping the flow for any desired time during the electrophoretic separation step, then restarting the flow to move the separated fractions out of the common exit. Provisions for permitting the recovery of separated sample fractions and for UV detection of biologicals have been made for possible later addition to the unit. More detailed discussions on each of the major subsections follows.

B. Electrophoresis Cell

Ground-based testing in prototype equipment was conducted on several innovations in cell design such as cells containing multiple inlets and multiple sample injections. These can be seen in Figures 8 and 9. These were used for some tests to ascertain the upper limit of sample input rates as part of the objective of increasing the throughput. The electrophoresis cell has inside dimensions of 33.6 cm x 5.08 cm x 0.5 cm with an active electrode length of 10.2 cm. The cell faces are glass-reinforced epoxy, which was chosen for its rigidity and high thermal conductivity (relative to most plastics). The entire assembly is contained within an aluminum frame

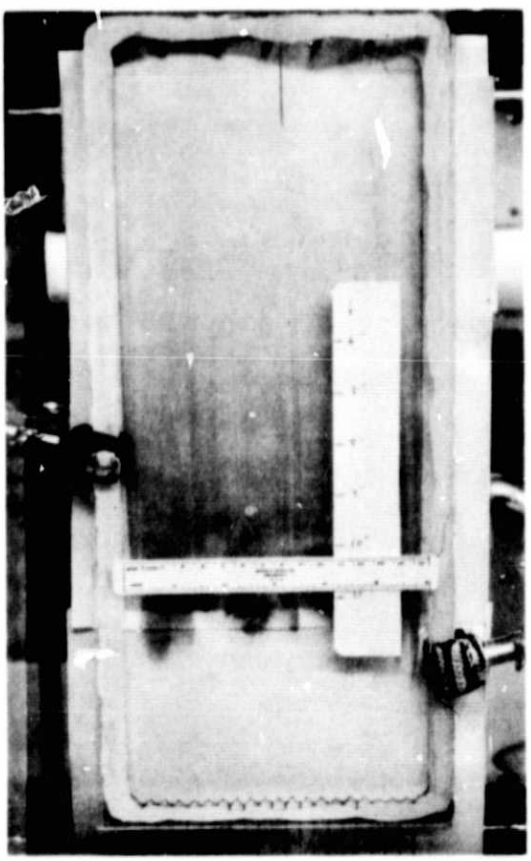


Figure 8. Fluid Flow in a Cell with Multiple Inlets

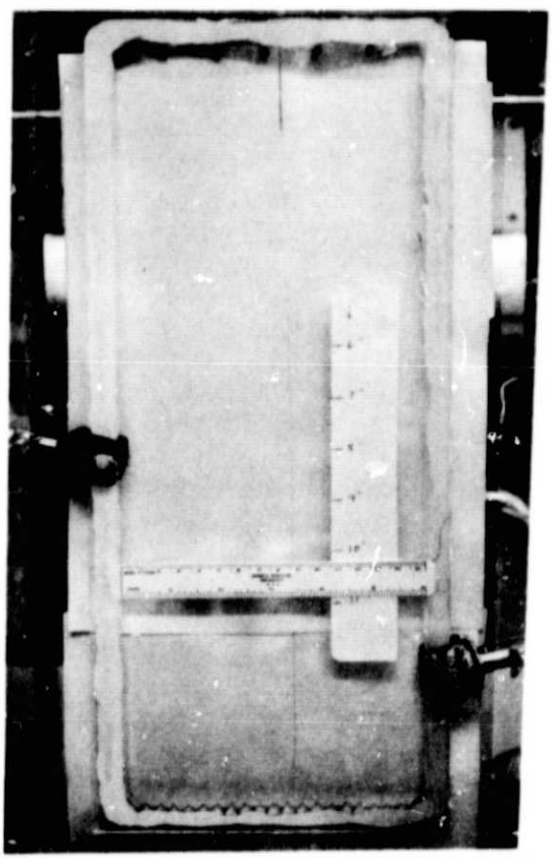


Figure 9. Sample Stream in a Cell with Multiple Inlets

which also serves as the cooling jacket in the lectrode area. Photographs of a cell devoid of plumbing and electrical connections are shown in Figures 10 and 11.

A duplicate of the engineering model cell is now in usage for determining the thresholds between unstable conditions due to terrestrial gravitational effects and stable conditions in space, which are also in the desired operating range. In other words, if it is desired for resolution or throughput reasons to operate the cell in the region where it is unstable on earth, it may be at conditions which require the microgravity of space to permit stable operation. The definition of some of these conditions is apparently required to gain a recommendation for a sounding rocket flight.

C. Fluid Systems

There are basically three fluid systems, each with a pump and provision for low-temperature operation.

1. Buffer
2. Coolant
3. Sample

The central feature of the buffer system is a reversible, positive displacement, piston pump with a capacity of 1625 ml shown in Figure 12. It is intended that in flight operation the buffer pump would be started ju t

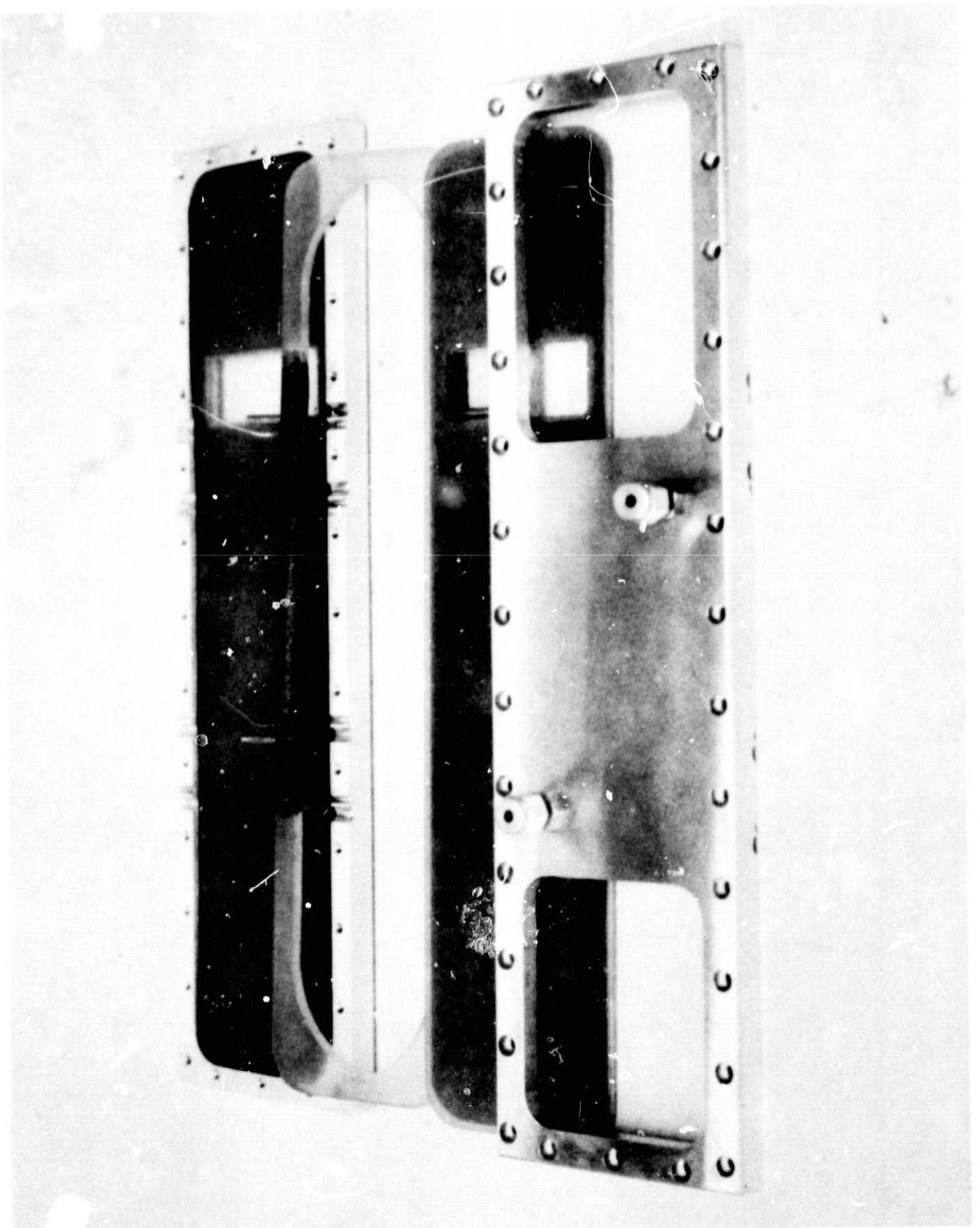


Figure 10. Electrophoresis Cell Components

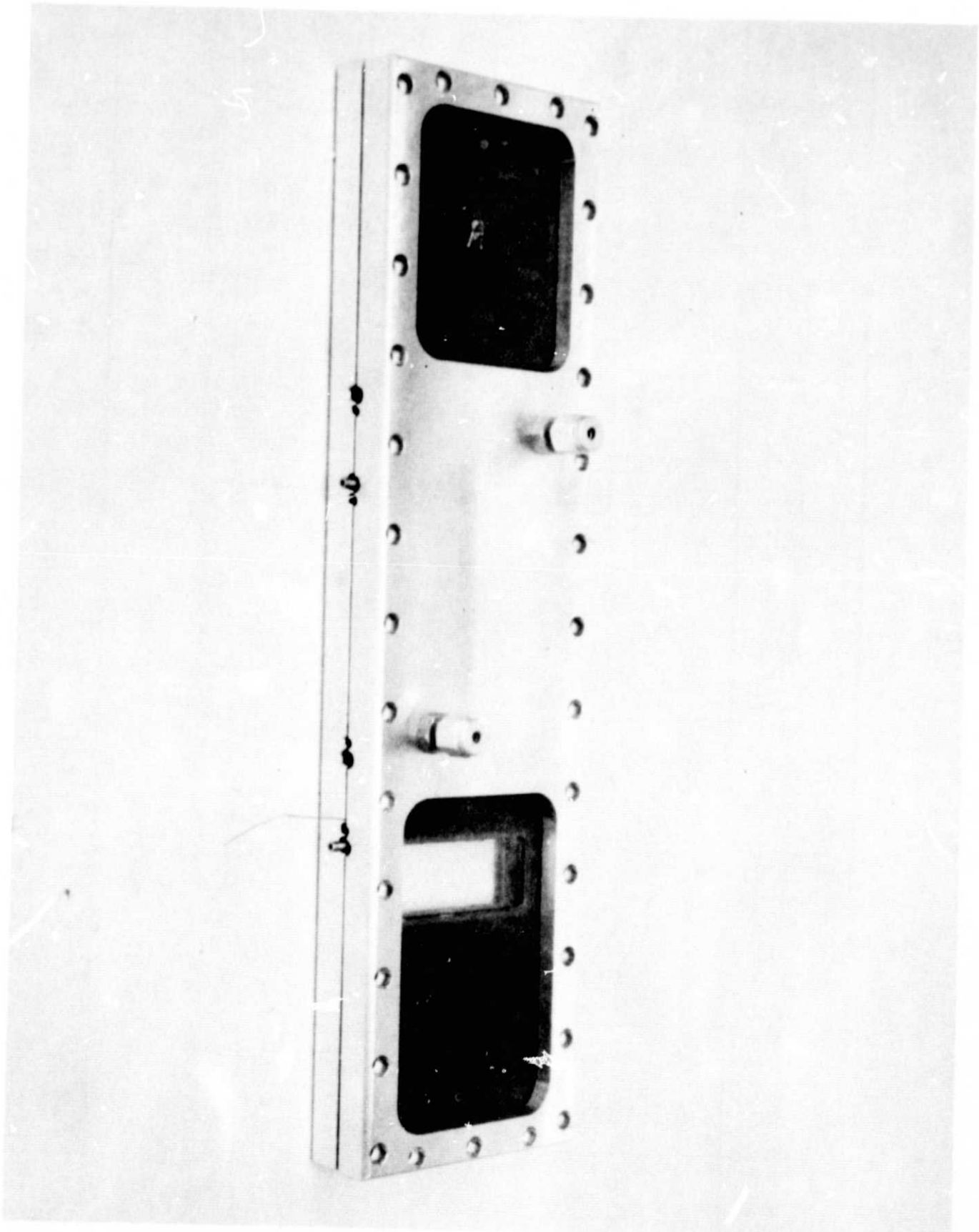


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Figure 11. Electrophoresis Cell Assembled

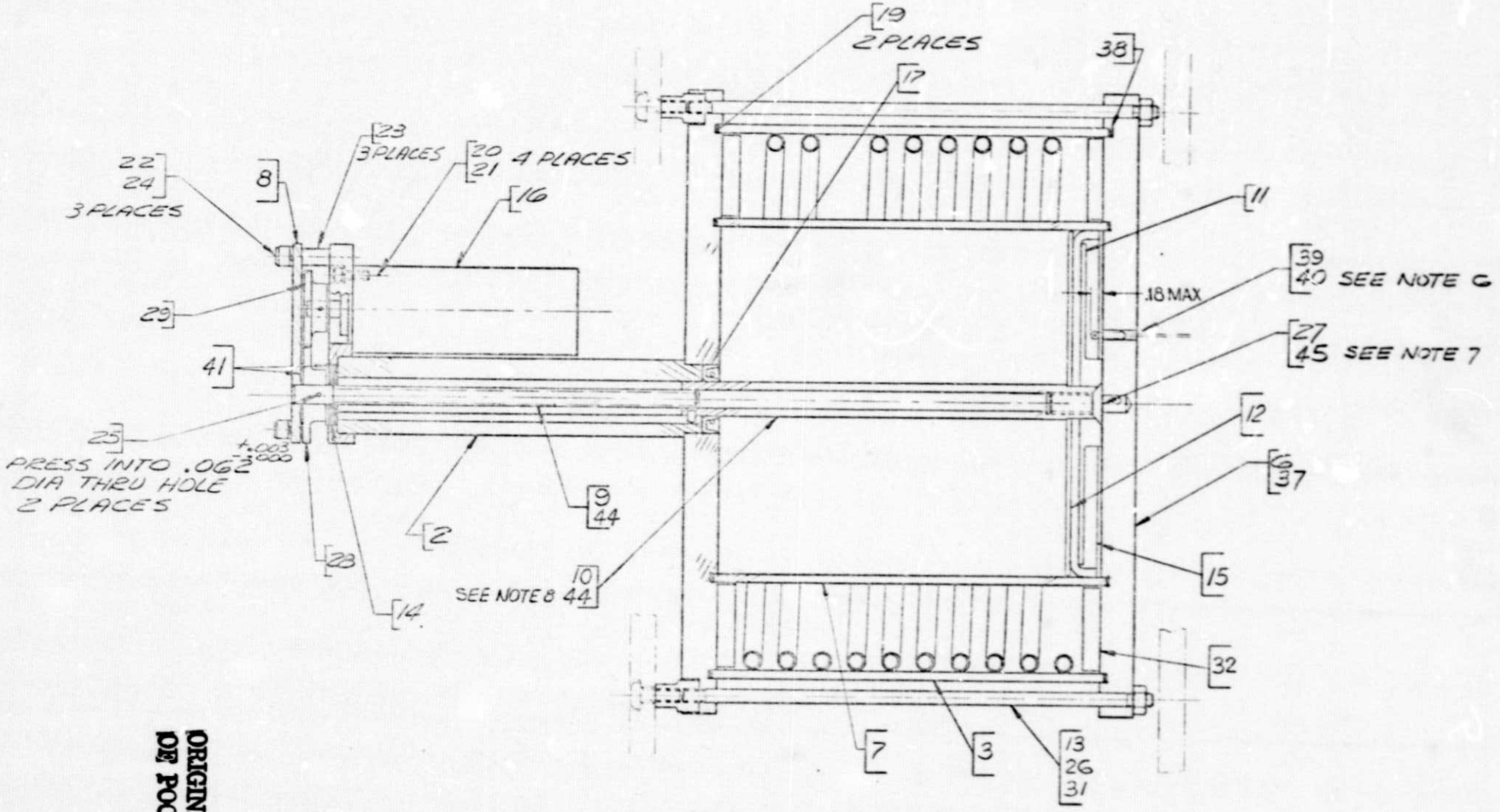

FIGURE 12. BUFFER PUMP

prior to launch to lower and equilibrate the cell temperature to about $4^{o}C$. It is designed therefore with enough capacity to provide this function as well as to permit the potential usage on the Aries rocket for 12-15 minutes of microgravity. To maintain essentially a constant center of gravity, the buffer (and spent sample) is returned to the low pressure side of the piston.

The coolant or temperature controllant is basically a fluid system for providing cooled ($4^{o}C$) water to the electrophoresis cell faces. The cooling capability for both this coolant and the buffer is supplied by prefrozen coolant located in an annular space around the buffer pump. It would also be started prior to flight to help to equilibrate the cell temperature.

The sample handling system is a motor driven cooled, insulated, syringe mounted on a bracket to permit easy installation on (and removal from) the experimental facility through the access door up to about an hour prior to flight. It is shown in Figure 13.

D. Data Acquisition System

The initial data acquisition system is aimed at demonstrating the primary objective of the program, namely improved resolution from a thick (5 mm) cell. It was therefore designed as a cross-sectional analyzer sytem with a Nikon F2 camera to record the images which are presumed

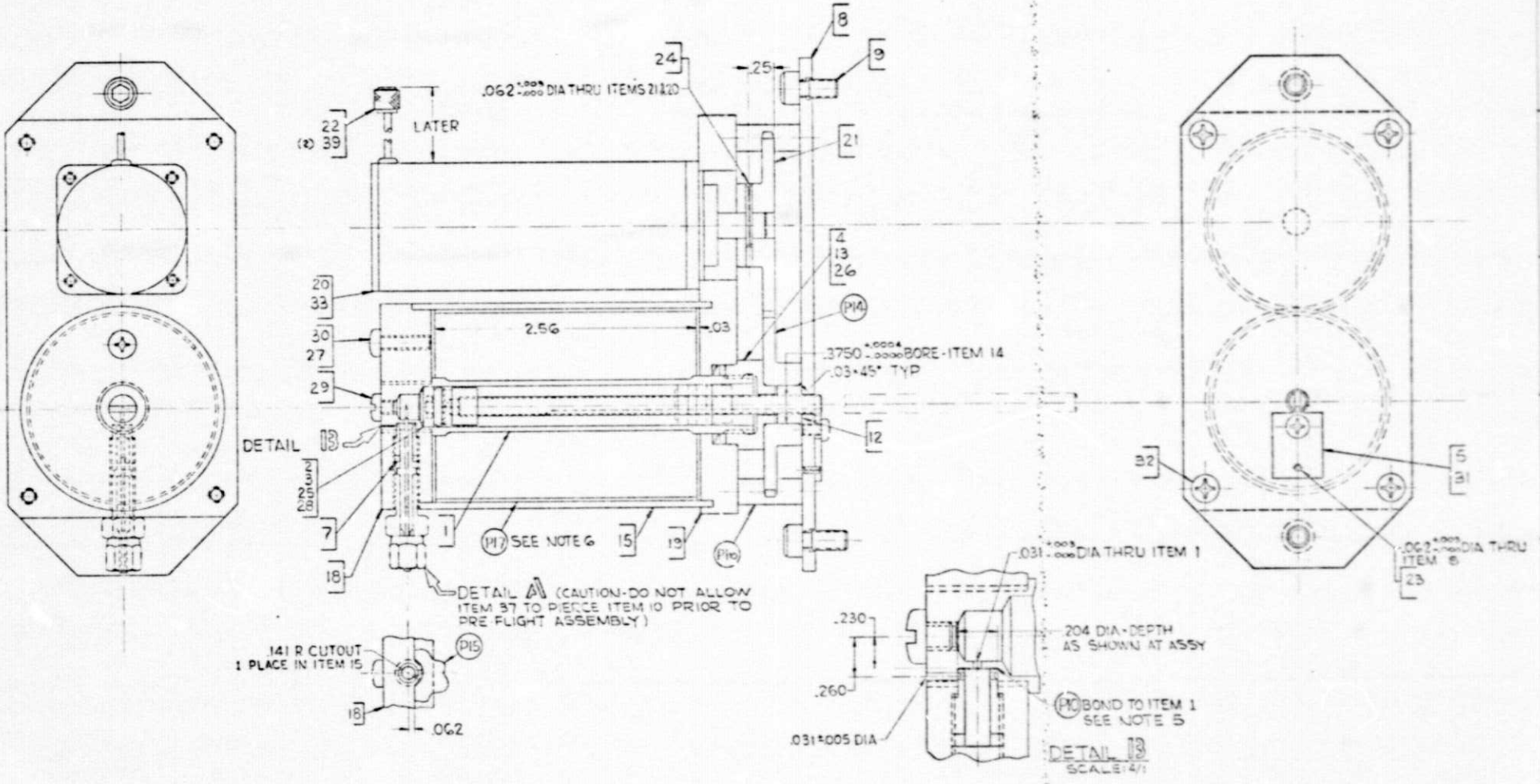

FIGURE 13. SAMPLE PUMP

to be from visible particles for the stated purpose.

In the expectation, however, that the facility will be used eventually for biologicals most of which are only or best observed in the UV spectrum, some provisions have been made to add that capability. Among the provisions are a fused silica envelope flash lamp and fused silica windows in the cell to permit generating and transmitting UV light respectively. In addition, some space and electronic circuitry are provided for adding a UV scanner to the system later.

All of these components, plus the electrophoresis cell, are mounted on the top structural plate shown in Figure 6. This permits the alignment and testing of the data acquisition system with little danger of later misalignment due to the flight environment, etc.

In addition to the potential future addition of a UV scanner for observing biologicals, some thought and provision for easily adding sample fraction recovery capability to the facility has been provided. This would primarily involve replacing the camera volume with a container for cold water from the coolant reservoir and means for holding the separated fractions in individual containers. This would place the recovered sample fractions just inside the access door from which they could be easily recovered after flight.